REMARKS

Status of the Claims and Amendment

Claims 1-2, 5, 12, 21, 27, and 30 have been amended. Claims 3 and 11 are canceled herewith without prejudice or disclaimer. Claims 4 and 9 were previously canceled. Claims 1-2, 5-8, 10, and 12-32 are all the claims pending in this application. Claims 1-3, 5-8 and 10-32 are rejected.

Claim 1 has been amended to even further clarify that the claimed plasmid comprises, as a regulatory unit, "a regulatory element for the expression of the recombinase characterised in that it comprises a gene coding for a sequence specific recombinase, a minicircle identification sequence for the identification and isolation of the minicircle and/or a miniplasmid identification sequence for the identification, isolation and removal of the miniplasmid and wherein a gene coding for a specific protein is inserted into the multiple cloning site," and that "upon induction of the expression of the sequence specific recombinase via the regulatory element" the units are arranged on the plasmid in such a way that the plasmid is divided into a miniplasmid and a minicircle. Support for the amendments to claim 1 may be found throughout the specification, for instance, at the paragraph bridging pages 9-10 to page 10, 2nd full paragraph, page 14, 2nd to 3rd full paragraph and claim 11.

Claims 2, has been amended to correct a clerical error, i.e., delete a repetitive phrase "a gene coding for a specific protein, preferably."

Claims 5 and 12 have been amended to change the claim dependency to claim.

Claim 21 has been amended to even further clarify that the kit is "characterized in that it comprises a separate plasmid carrying the inducible lysis gene." Support for the amendment to

claim 21 may be found throughout the specification, for instance, at page 13, last paragraph to page 14, 1st full paragraph.

Claim 27 has been amended to even further clarify that the recombinase is expressed "upon induction of the regulatory element so that plasmids and minicircles are produced." Support for the amendments to claim 1 may be found throughout the specification, for instance, at pages 9-10 to page 10, 2nd full paragraph and page 14, 2nd to 3rd full paragraph.

The specification at pages 17, 18, 20 and 21 have been amended to include sequence identifiers in response to the objections to the specification.

No new matter is added.

Claim of Priority

Applicants thank the Examiner for acknowledging Applicants' claim to foreign priority, and for indicating that certified copy of the priority document, Austrian Patent Application No. A700/2003 has been received.

Information Disclosure Statement

Applicants thank the Examiner for returning a signed and initialed copy of the PTO Form SB/08 that accompanied the Information Disclosure Statement filed July 24, 2009.

However, the two (2) PTO Forms SB/08 submitted to the Office on November 8, 2008, remain outstanding. Applicants respectfully request the Examiner consider the references cited therein by returning signed and intialed PTO SB/08 forms submitted therewith.

Response to Objections to the Specification

On page 2 of the Office Action, the Examiner objects to the specification for failing to comply with the requirements of 37 C.F.R. § 1.821 through 1.825. The specification fails to provide SEQ ID NOs. for the nucleotide sequences disclosed on pages 17-18 and 20-21.

In response, and solely to advance prosecution of the present application, pages 17-18 and 20-21 have been amended to include sequence identifiers.

Withdrawal of the grounds of objection is respectfully requested.

Claims 1-3, 5-8, 10-32 Are Enabled Under 35 U.S.C. § 112

Claims 1-3, 5-8, 10-32 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement.

The Examiner asserts that the as-filed specification teaches plasmid(s) which upon the conditional expression of ParA recombinase (induced via arabinose) results in a miniplasmid comprising i) origin of replication and ii) a marker gene (drug resistance gene), whereas the minicircle comprises i) gene encoding ParA resolavse operavbly linked to araC repressor/inducer, ii) identification sequence (lac operator) and ii) an anchoring peptide (lacl-L fusion protein) see Figure 5; and Spec. Pages 22-25).

However, the Examiner asserts that the speciation fails to disclose any other plasmid structure which would enable one skilled in the art to practice the invention as claimed in claim 1 in a predictable fashion without further undue amount of experimentation. Specifically, the Examiner states that the specification fails to disclose any plasmid vector which comprises "functional units arranged in such a way that the plasmid is divided into a miniplasmid and a minicircle upon expression of the sequence specific recombinase (Para resolvase), said miniplasmid comprising the prokaryotic origin of replication, the marker sequence and the gene for the sequence specific recombinase and said minicircle comprising the multiple cloning site."

Furthermore, with regard to the minicircle identification sequence used for the identification and isolation of the minicircles, the Examiner asserts that besides lacO that binds to lacl-L (hybrid fusion anchoring peptide: Lacl+MS2L), the specification fails to disclose any

other identification/isolation system, which would enable one skilled din the art to practice the invention as claimed. Also, the Examiner asserts that besides inducing bacterial lysis for isolation of minicircles by co-transfecting another plasmid (E-specific/temperature-specific bacterial lysis), the specification fails to disclose any other bacterial lysis system(inducible), which would enable one skilled in the art to practice the invention as claimed.

Initially, Applicants note that solely to advance prosecution of the present application, claim 1 (from which claims 2-3, 5-8, and 10-32 directly or indirectly depend) has been amended to even further clarify that the claimed plasmid comprises, as a regulatory unit, "a regulatory element for the expression of the recombinase characterised in that it comprises a gene coding for a sequence specific recombinase, a minicircle identification sequence for the identification and isolation of the minicircle and/or a miniplasmid identification sequence for the identification, isolation and removal of the miniplasmid and wherein a gene coding for a specific protein is inserted into the multiple cloning site," and that "upon induction of the expression of the sequence specific recombinase via the regulatory element" the units are arranged on the plasmid in such a way that the plasmid is divided into a miniplasmid and a minicircle.

Claims 3 and 11 have been canceled, rendering the rejection moot with regard to claims 3 and 11.

In response, Applicants note that, "[d]etailed procedures for making and using the invention may not be necessary if the description of the invention itself is sufficient to permit those skilled in the art to make and use the invention" (see M.P.E.P. §2164) "[a]s long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim" (see M.P.E.P. §2164.01(b)).

In the present case, for at least the reasons discussed below, one of ordinary skill in the art would be enabled to make and use the presently claimed plasmid without undue experimentation, based on the disclosure in the specification, as well as the knowledge available in the art and technical knowledge possessed by one of ordinary skill in the art.

First, in response to the Examiner's assertions that the specification fails to disclose any plasmid structure enabling the skilled person to practice the invention as presently claimed, *i.e.*, wherein the functional units are arranged in such a way that the plasmid is divided into (i) a miniplasmid comprising the prokaryotic origin of replication, the marker sequence and the gene for the sequence specific recombinase and (ii) a minicircle comprising the multiple cloning site; Applicants note the following. As shown in Figure 5[1], starting from the plasmid pHCNparA, one of ordinary skill in the art would have understood based on the description in the specification, that they would only have to change the position of one resolution site and integrate a multiple cloning site into said construct in order to make claimed subject matter, as the remaining elements are already correctly arranged in the plasmid of Figure 5. Further, guidance on how this resolution site should be arranged is provided on page 6, third paragraph of the description stating that "*[...] which means that in fact the sequences which are recognized by the recombinase should flank the multiple cloning site which will then provide the minicircle.*" In this respect, as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied. M.P.E.P. §2164.01(b).

[1] All citations given herein refer to the originally filed application.

Additionally, in view of the general technical knowledge in the art at the time the present application was filed, one of ordinary skill in the art would have been clearly enabled to rearrange the resolution site and integrate the multiple cloning site into pHCNparA as shown in Figure 5 of the present patent application and therefore would, following the guidance given in the description, arrive at the presently claimed subject invention starting from Figure 5 without undue experimentation.

This is further evidenced by the additional experimental data provided herewith; which demonstrates, as shown in Figure 1 (attached herewith), how the claimed subject-matter may be generated starting from the pHCNparA plasmid as shown in Figure 5 of the description.

Similarly, Figure 2 (attached herewith) of the additional experimental data attached herewith shows that a plasmid wherein a gene of interest has been inserted in the multiple cloning site will divide into a minicircle and a miniplasmid as presently claimed.

Hence, based on the description in the as-filed specification, and as further evidenced by the experimental data submitted herewith, one of ordinary skill in the art would be enabled to make and use the presently claimed invention without undue experimentation. In this regard, the Board of Patent Appeals and Interferences has stated that "[t]he amount of experimentation to practice the full scope of the claimed invention might have been extensive, but it would have been routine. The techniques necessary to do so were well known to those skilled in the art." *Ex parte Kubin* (B.P.A.I. 2007). Further, "[t]he fact experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation." M.P.E.P. §2164.01. In other words, the "[t]he scope of enablement varies inversely with the degree of predictability involved, but even in unpredictable arts, a disclosure of every operable species is not required." M.P.E.P. §2164.03.

Second, the Examiner appears to assert, *inter alia*, referring to post-published documents — that the stringent control of repression and expression of (i) the recombinase, (ii) the inducible lysis gene and (iii) the membrane anchoring peptide is an essential requirement for the success of the present invention, and that without a specific arrangement of individual functional units, it would require undue experimentation to repress/express the desired units to make and use the claimed invention. In response, Applicants note the following.

(i) Recombinase expression

With regard to the Examiner's assertions concerning the control of the recombinase expression, Applicants note that solely to advance prosecution of the present application, amended claim 1 requires a regulatory element for the expression of the recombinase in the plasmid and that the plasmid is divided into a miniplasmid and a minicircle upon induction of the expression of the recombinase via the regulatory element. Similarly, claims 27 and 30 require that the recombinase be expressed upon induction of the regulatory element. Further, as described, for instance, at page 9, last paragraph to page 10, third paragraph of the description, various regulatory elements are known to one of ordinary skill in the art.

(ii) Lysis gene

With respect to the Examiner's assertions that the claimed invention does not require the control of repression and expression of the lysis gene, claim 21 as amended is directed to an inducible lysis gene.

With regard to the Examiner's assertions that it is not clear whether the inducible lysis gene is expressed from the parental plasmid or is co-transfected on a separate plasmid, Applicants note that as described at the last paragraph on page 13 of the specification, the inducible lysis gene may be either on the parental plasmid or be provided on a separate plasmid,

and one of ordinary skill in the art would clearly know how to perform both based on the guidance in the specification and the technical knowledge possessed by one of ordinary skill in the art.

In this respect, the as-filed specification provides guidance with regard to the expression control of the inducible lysis gene. For instance, the last paragraph on page 13 describes that once the recombinase has produced a minicircle and a miniplasmid the lysis gene can be activated. This is also shown in Example 3 of the present application and in particular, on page 26, last paragraph stating that after the induction of the expression of the lacI-L' hybrid gene and the ParA resolvase gene the E-lysis was induced by shifting the temperature, as the lysis gene is under the control of a temperature sensitive promoter/repressor system. Hence, the as-filed specification provides sufficient guidance with respect to expression control of the lysis gene.

(iii) Expression of the hydrophobic anchoring peptide

The Examiner also asserts that the stringent control of repression and expression of the hydrophobic anchoring peptide is of high importance, as the hydrophobic anchoring peptide would easily impair the DNA replication or DNA binding functionalities.

Applicants note that in the examples of the as-filed specification, the gene coding for the hydrophobic anchoring peptide (L') is fused to the C-terminal end of the gene coding for the lacI repressor protein. This hybrid protein is under the control of the same promoter as the ParA resolvase (cf. *e.g.*, Figure 5 and the last paragraph on page 24 of the description). Upon induction of this promoter, a bicistrionic mRNA is produced, coding for the ParA resolvase as well as for the LacI-L' fusion protein. Hence, both proteins are under the control of said promoter and are simultaneously expressed upon its induction. Due to its physicochemical properties, the hydrophobic membrane anchoring peptide fused to the LacI protein interacts with

the hydrophobic lipid bilayer of the cytoplasmic membrane, thereby immobilizing the hybrid protein in the bacterial membrane. Therefore, the hydrophobic peptide itself does neither interact with the minicircle identification sequence (or any other DNA) nor does it impair DNA replication or DNA binding functionalities. None of the post-published documents cited by the Examiner provide any suggestion that the binding of a repressor would have adverse effects on the DNA replication. In that regard, it has never been observed or reported in the art, that the binding of the LacI-L' hybrid protein to the minicircle identification sequence has an influence on the protein E driven process of bacterial ghost formation. The DNA binding domain of the LacI-L' protein binds reversibly to the minicircle identification sequence. Therefore, the DNA minicircles anchored to the bacterial ghosts may be dissected by standard processes and be isolated subsequently, as stated on page 26 first paragraph of the as-filed specification. Minicircle-DNA produced with the bacterial ghost system can therefore be subjected to subsequent affinity purification without any problems with respect to DNA binding.

Finally, with regard to the minicircle identification sequence, the Examiner asserts that only the lacO/LacI-L' system is disclosed as a minicircle identification sequence in the present application. In response, Applicants note that as described at page 7, second paragraph to page 9, first paragraph of the as-filed specification, various minicircle/miniplasmid identification sequences are described, and the lacO/LacI-L' system is described as a preferred embodiment (cf. in particular page 8, lines 25-34 disclosing various DNA binding proteins for the protein DNA interaction chromatography.

Thus, for the reasons set forth above, one of ordinary skill in the art would be enabled, base on the guidance provided in the as-filed specification as well as the knowledge available in

the art and possessed by one of ordinary skill in the art, to make and use the presently claimed invention.

Reconsideration and withdrawal of the rejection under 35 U.S.C. § 112, first paragraph, is respectfully requested.

Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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